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(54) Title: COTTON ALPHA-GLOBULIN PROMOTER FOR SEED-SPECIFIC EXPRESSION OF TRANSGENES

(57) Abstract: The present invention is directed to 5' regulatory regions of a cotton seed-specific gene, α-globulin. The 5' regulatory region, or parts thereof, when operably linked to either the coding sequence of a native gene, heterologous gene or a sequence or complementary sequence in a plant seed. The regulatory regions are useful in expression cassettes and expression vectors for the trnasformation of plants. Also provided are methods of modulating the levels of a native or heterologous gene such as a fatty acid synthesis or lipid metabolism gene by transforming a plant with the subject expression cassettes and expression vectors.

Cotton α-Globulin Promoter For Seed-Specific Expression of Transgenes

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SPECIFICATION

FIELD OF THE INVENTION

This invention is in the field of transgene expression. More particularly, the invention is in the field of gene transcription promoter elements useful for transgene expression in plants.

BACKGROUND OF THE INVENTION

Seed-specific transgene expression is required for a number of applications utilizing genetic engineering. These include transgenic means to improve seed nutritional quality by manipulating flux through metabolic pathways (Hitz et al., 1995; Kinney, 1996; Shintani and DellaPenna, 1998; Goto et al., 1999) and for the production of novel compounds of industrial or pharmaceutical value (Cahoon et al., 2000) in a convenient package, the seed. Some of these transgenic traits may require expression of more than one transgene in the developing seed (Ye et al., 2000). In other cases, metabolic engineering to improve seed quality may require over-expression and/or suppression of various genes during seed development. Each step will require a promoter of appropriate strength depending on the desired degree of over-expression or suppression. In addition, a promoter with appropriate developmental timing may also be required. Even if the same degree of expression of more than one gene is required, it is not advisable to use the same promoter for multiple introduced genes. In some cases of high copy number integration of transgenes, promoter homology can lead to gene silencing (Vaucheret, 1993; Brusslan and Tobin, 1995; Park et al., 1996).

Seed storage proteins are expressed at high levels during seed development, and their expression is tightly controlled both spatially and temporally in the developing seed. Therefore, regulatory sequences from genes encoding seed storage proteins represent a valuable source of promoters that can be utilized to drive the expression of transgenes in a seed-specific manner. The promoters from soybean β-conglycinin genes (Barker et al., 1988; Chen et al., 1988; Lessard et al., 1993), French bean phaseolin gene (Bustos *et al.*, 1989,1991; Kawagoe et al., 1994), sunflower helianthinin gene (Bogue *et al.*, 1990; Nunberg et al., 1995), and the carrot *Dc3* promoter (Seffens et al., 1990; Kim et al., 1997) are examples of some of the well-characterized seed-specific promoters from dicots. Despite this array of other promoters available, the problems of expression levels and gene silencing are still an issue. Thus, it is clear that there will be an increasing need for promoters of varying strengths from more than one source to meet the future demands to regulate expression of one or more transgenes in seeds.

SUMMARY OF THE INVENTION

In response to the continuing need for novel promoter elements in the field of plant transgenics, an 1144 bp 5' regulatory region comprising an 1108 bp promoter sequence and a 36 bp

5' transcribed, untranslated sequence from a cotton α-globulin gene was isolated and functionally characterized. Globulins are principal seed storage proteins of cotton and constitute about 60% of total proteins at seed maturity (Dure, 1989). In cotton, two α-globulin genes, gene A and gene B, encode proteins of molecular weight 48 and 51 kDa, respectively (Chlan et al., 1987).

The present invention provides for transgene expression in plants using an α-globulin gene promoter according to the teachings disclosed herein. The α-globulin gene promoter disclosed in this specification addresses the continuing need for novel promoter elements in the field of plant transgenics. In at least one embodiment, the transgene expression is seed specific as defined by the teachings disclosed herein.

In one embodiment, the transgene expression is performed using a promoter DNA containing the 1108 bp α-globulin gene promoter sequence. In a different embodiment, the transgene expression uses a promoter DNA containing additional sequence that does not interfere with the promoter function of the 1108 bp sequence. In yet another embodiment, the transgene expression uses a promoter DNA containing only part of the 1108 bp sequence such that the promoter function capacity of the partial sequence is functionally similar to the full 1108 bp sequence during seed and plant development.

In one embodiment, an α-globulin gene promoter is used to drive the transcription of an RNA encoding a polypeptide for expression in seed. In a further embodiment, the RNA encodes a polypeptide with commercial value such as an enzyme, an antibody, or peptides for vaccines. In yet another embodiment, an α-globulin gene promoter causes the expression of protein(s) that prevent seed germination. In a further embodiment, an α-globulin gene promoter causes the transcription of an RNA for a polypeptide that improves the nutritional quality of a seed.

In another embodiment, an α-globulin gene promoter drives the transcription of RNAs with desired properties such as an antisense RNA or a ribozyme. In one particular embodiment, the antisense RNA is complementary to the RNA encoding the enzyme involved in the biosynthesis of the toxin, Gossypol expressed in cotton. In another embodiment, the antisense RNA is complementary to RNA for one or more enzymes involved in fatty acid synthesis. In an advantageous embodiment, such RNAs can target viral genomes or transcripts to prevent or reduce disease. In another advantageous embodiment, such RNAs can target and control the expression of endogenous transcripts.

In another embodiment, an α-globulin promoter drives the transcription of a DNA sequence whose transcript will form a hairpin structure that mediates post-transcriptional gene silencing of a native gene through RNA interference. In a different embodiment, an α-globulin gene promoter causes the transcription of an RNA encoding an endogenous protein to silence the gene for that protein through the mechanism of cosuppression (U.S. Pat. No. 6,100,450; Column 12, lines 42-60).

In yet another embodiment, an α-globulin gene promoter drives the expression of transcripts that regulate the fatty acid content of dicot seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

A more particular description of the invention, briefly summarized above, may be had by reference to the embodiments thereof which are illustrated in the appended drawings and described herein. It is to be noted, however, that the appended drawings illustrate only some embodiments of the invention and are therefore not to be considered limiting of its scope, because the invention may admit to other equally effective embodiments.

FIG. 1 shows the α-globulin promoter sequence and reporter gene construct. **A.** Nucleotide sequence of the promoter region. **B.** T-DNA of the binary vector pBIAGPGUS used in an embodiment of the present invention.

FIG. 2 shows the histochemical localization of GUS activity in developing embryos from stably transformed tobacco, cotton, and *Arabidopsis* plants and in germinating *Arabidopsis* seedlings. **A-E**. Activity in embryos from T1-homozygous tobacco plant: **A.** embryo 9 days post anthesis (dpa), **B.** embryo 10 dpa, **C.** embryo 13 dpa, **D.** embryo 17 dpa, **E.** mature embryo from dry seed; **F-N**. GUS activity in embryos from T1-homozygous cotton plant: **F.** high magnification image of embryo 16 dpa showing the beginning of GUS activity, **G.** same embryo at lower magnification, **H.** embryo 18 dpa, **I.** embryo 19 dpa, **J.** embryo 20 dpa, **K.** embryo 25 dpa, **L.** embryo 30 dpa, **M.** embryo 40 dpa, **N.** embryo isolated from dry seed that has been cut through the middle showing the staining in the radicle and hypocotyl regions; **O.** embryo from a null segregant cotton seed; **P-T:** GUS activity in seeds of *Arabidopsis thaliana:* **P.** torpedo stage embryo (4 dpa), **Q.** walking-stick stage embryo (5 dpa), **R.** upturned-U stage embryo (7 dpa), **S.** partially mature embryo (9 dpa), **T.** mature embryo from dry seed; **U-Y**: GUS activity during seed germination in transgenic *Arabidopsis:* **U.** embryos isolated from dry seeds, **V.** one-day-old seedling, **W.** 3-day-old seedling, **X.** 5-day-old seedling, **Y.** 8-day-old seedling. Bars: A-E, P-T = 100 μm; F-O, U-Y = 1 mm.

FIG. 3 shows the developmental regulation of GUS expression by the α-globulin promoter in tobacco. **A.** Developmental regulation of GUS expression by the α-globulin promoter in tobacco seeds. **B.** GUS specific activity in tobacco seedlings during germination at different days post imbibition.

FIG. 4 shows the developmental regulation of GUS expression by the α-globulin promoter in cotton embryos. GUS specific activity (closed circle) and total protein (open circle) in extracts from developing cotton embryos as a function of days post anthesis (dpa).

FIG. 5 shows GUS activity in seeds from independent transgenic lines of T0 tobacco, T1 *Arabidopsis*, and T0 cotton.

FIG. 6 shows GUS specific activity in individual embryos isolated from seeds from a T1

homozygous and seeds from a single T1 hemizygous cotton plant.

FIG. 7 shows that the α-globulin promoter drives antisense expression of the δ-12 desaturase gene from cotton to increase the levels of oleic acid in different transgenic lines of cottonseeds. C: non-transformed control plant. O: Cottonseed oil (Supelco, Bellefonte, USA).

FIG. 8 shows the fatty acid levels of four lines of high-oleate cotton seeds at the individual seed level. Oleic acid (triangle) and linoleic acid (open square) levels in individual T1 seeds from four T0 lines H50-2 (A), H41-1 (B), H4-2 (C) and H42-2 (D). Numbers in the X axis represent individual seeds. P: 30 pooled seeds.

FIG. 9 shows the fatty acid levels in T2 cotton seeds germinated from two of the high-oleate trangenic lines. Oleic acid (triangle) and linoleic acid (open square) levels in T2 seeds from T1 plants of two high oleic acids lines H50-2 (A) and H41-1 (B). A pooled sample of 30 T2 seeds from 15 different T1 plants of each line was tested.

FIG. 10 shows the 336 bp sequence underlined in FIG. 1 and separately set forth in SEQ NO ID:3

FIG. 11 shows the 1108 bp sequence present in FIG. 1 and separately set forth in SEQ NO ID:2

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

The discussion and examples which follow detail the best known method for performing the invention. It will be recognized that variations of this method may include heterologous or native genes or constructs or modifications to the regulatory sequences, dependent upon the target plant species and the traits to be transferred into the target plants. Cotton, tobacco, and *Arabidopsis* species were chosen as the target plants in the following examples; however, the use of the cotton α-globulin promoter and method of driving expression of native or heterologous genes outlined below is adaptable to other plants without significant experimentation or deviation from the spirit and scope of this invention.

Globulins are known to be the most prevalent seed storage proteins of dicotyledonous plants (Borroto and Dure, 1987) and their regulatory sequences potentially are a useful source of promoters that can be utilized to confer strong seed-specific expression of transgenes in a wide range of dicot species.

The present invention provides isolated nucleic acids encoding 5' regulatory regions from the seed-specific cotton alpha globulin B (AG) gene, designated as either α-globulin promoter or AGP. In accordance with the present invention, the subject 5' regulatory regions, when operably linked to either a coding sequence of a heterologous gene or a sequence complementary to a native plant gene, direct expression of the coding sequence or complementary sequence in a plant seed. The AG 5' regulatory regions of the present invention are useful in the construction of expression cassettes which comprise in the 5' to 3' direction, a subject AG 5' regulatory region, a heterologous gene or sequence complementary to a native plant gene under control of the regulatory region and a 3' termination sequence. Such an expression cassette can be incorporated into a variety of

autonomously replicating vectors in order to construct an expression vector.

Modifications to the AG regulatory regions, including the individual promoters and 5' untranslated regions as set forth in SEQ NO ID:1 and SEQ NO ID:2, which maintain the characteristic property of directing seed-specific expression, are within the scope of the present invention. Such modifications include insertions, deletions and substitutions of one or more nucleotides.

Confirmation of seed-specific 5' regulatory regions which direct seed-specific expression and modifications or deletion fragments thereof, can be accomplished by construction of transcriptional and/or translational fusions of specific sequences with the coding sequences of a heterologous gene, transfer of the chimeric gene into an appropriate host, and detection of the expression of the heterologous gene. The assay used to detect expression depends upon the nature of the heterologous sequence. For example, reporter genes, exemplified by chloramphenicol acetyl transferase and β-glucuronidase (GUS), are commonly used to assess transcriptional and translational competence of chimeric constructions. Standard assays are available to sensitively detect the reporter enzyme in a transgenic organism. The β-glucuronidase (GUS) gene is useful as a reporter of promoter activity in transgenic plants because of the high stability of the enzyme in plant cells, the lack of intrinsic β-glucuronidase activity in higher plants and availability of a quantitative fluorimetric assay and a histochemical localization technique. Jefferson et al. (1987b) EMBO J 6; 3901-3907 have established standard procedures for biochemical and histochemical detection of GUS activity in plant tissues. Biochemical assays are performed by mixing plant tissue lysates with 4-methylumbelliferyl-β-D-glucuronide, a fluorimetric substrate for GUS, incubating one hour at 37°C, and then measuring the fluorescence of the resulting 4-methyl-umbelliferone. Histochemical localization for GUS activity is determined by incubating plant tissue samples in 5-bromo-4-chloro-3-indolyl-glucuronide (X-Gluc) for about 18 hours at 37°C and observing the staining pattern of X-Gluc. The construction of such chimeric genes allows definition of specific regulatory sequences and demonstrates that these sequences can direct expression of heterologous genes in a seed-specific manner.

An aspect of the invention is directed to expression cassettes and expression vectors (also termed herein "chimeric genes") comprising a 5' regulatory region or portion thereof from an AG gene which direct seed specific expression operably linked to the coding sequence of a heterologous gene such that the regulatory element is capable of controlling expression of the product encoded by the heterologous gene. The heterologous gene can be any gene other than AG. If necessary, additional regulatory elements from genes other than AGP or parts of such elements sufficient to cause expression resulting in production of an effective amount of the polypeptide encoded by the heterologous gene are included in the chimeric constructs.

Accordingly, the present invention provides chimeric genes comprising sequences of the AG 5' regulatory region that confer seed-specific expression which are operably linked to a sequence

encoding a heterologous gene such as a lipid metabolism enzyme. Examples of lipid metabolism genes useful for practicing the present invention include lipid desaturases such as δ6-desaturases, δ12-desaturases, δ15-desaturases and other related desaturases such as stearoyl-ACP desaturases, acyl carrier proteins (ACPs), thioesterases, acetyl transacylases, acetyl-coA carboxylases, ketoacyl-synthases, malonyl transacylases, and elongases. Such lipid metabolism genes have been isolated and characterized from a number of different bacteria and plant species. Their nucleotide coding sequences as well as methods of isolating such coding sequences are disclosed in the published literature and are widely available to those of skill in the art.

The chimeric genes of the present invention are constructed by ligating a 5' regulatory region or part thereof, of a AG genomic DNA to the coding sequence of a heterologous gene. The juxtaposition of these sequences can be accomplished in a variety of ways. In one embodiment, the order of sequences in a 5' to 3' direction, is an AG promoter, a coding sequence, and a termination sequence. In a preferred embodiment, the order of the sequences in a 5' to 3' direction is an AG promoter, an AG untranslated region, a coding sequence, and a termination sequence which includes a polyadenylation site.

Standard techniques for construction of such chimeric genes are well known to those of ordinary skill in the art and can be found in references such as Sambrook et al. (1989). A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments. One of ordinary skill in the art recognizes that in order for the heterologous gene to be expressed, the construction requires at least a promoter and signal for efficient polyadenylation of the transcript. Accordingly, the AG 5' regulatory region that contains the consensus promoter sequence known as the TATA box can be ligated directly to a promoterless heterologous coding sequence.

The restriction or deletion fragments that contain the AG TATA box are ligated in a forward orientation to a promoterless heterologous gene such as the coding sequence of β-glucuronidase (GUS). The skilled artisan will recognize that the subject AG 5' regulatory regions and parts thereof, can be provided by other means, for example chemical or enzymatic synthesis.

The 3' end of a heterologous coding sequence is optionally ligated to a termination sequence comprising a polyadenylation site, exemplified by, but not limited to, the nopaline synthase polyadenylation site, or the octopine T-DNA gene 7 polyadenylation site. Alternatively, the polyadenylation site can be provided by the heterologous gene. Or, 3' UTR which contain RNA localization signal ("zipcode") sequences. These determinants may be important in seed expression systems.

The present invention also provides methods of increasing the expression of a native gene or expressing heterologous genes in plant seeds. In accordance with such methods, the subject expression cassettes and expression vectors are introduced into a plant in order to effect expression of

a heterologous gene. For example, a method of producing a plant with increased levels of a product of a fatty acid synthesis or lipid metabolism gene is provided by transforming a plant cell with an expression vector comprising an AG 5' regulatory region or portion thereof, operably linked to a fatty acid synthesis or lipid metabolism gene and regenerating a plant with increased levels of the product of said fatty acid synthesis or lipid metabolism gene.

Another aspect of the present invention provides methods of reducing levels of a product of a gene which is native to a plant which comprises transforming a plant cell with an expression vector comprising a subject AG 5' regulatory region or part thereof, operably linked to a nucleic acid sequence which is complementary to the native plant gene. In this manner, levels of endogenous product of the native plant gene are reduced through the mechanism known as antisense regulation. Thus, for example, levels of a product of a fatty acid synthesis gene or lipid metabolism gene are reduced by transforming a plant with an expression vector comprising a subject AG 5' regulatory region or part thereof, operably linked to a nucleic acid sequence which is complementary to a nucleic acid sequence coding for a native fatty acid synthesis or lipid metabolism gene.

The present invention also provides a method of cosuppressing a gene which is native to a plant which comprises transforming a plant cell with an expression vector comprising a subject 5' AG regulatory region operably linked to a nucleic acid sequence coding for the native plant gene. In this manner, levels of endogenous product of the native plant gene are reduced through the mechanism known as cosuppression. Thus, for example, levels of a product of a fatty acid synthesis gene or lipid metabolism gene are reduced by transforming a plant with an expression vector comprising a subject AG 5' regulatory region or part thereof, operably linked to a nucleic acid sequence coding for a native fatty acid synthesis or lipid metabolism gene native to the plant. Although the exact mechanism of cosuppression is not completely understood, one skilled in the art is familiar with published works reporting the experimental conditions and results associated with cosuppression (Napoli et al. 1990; Van der Krol 1990).

The present invention also provides a method for regulating expression of a native gene which comprises transforming a plant cell with an expression vector comprising a subject 5' AG regulatory region operably linked to a nucleic acid sequence coding for sense part linked to the antisense part of the native plant gene. Transcript from such a construct having self-complementary arms forms a double-stranded RNA, hairpin structure and causes the degradation of transcripts from the native gene leading to post-transcriptional gene silencing (Waterhouse et al., 1998; Wang and Waterhouse, 2000; Chuang and Meyerowitz, 2000). This is similar to RNA-interference mechanisms.

To provide regulated expression of the heterologous or native genes, plants are transformed with the chimeric gene constructions of the invention. Methods of gene transfer are well known in the art. The chimeric genes can be introduced into plants by leaf disk transformation-regeneration

procedure as described by Horsch et al. (1988). Other methods of transformation such as protoplast culture can also be used and are within the scope of this invention. In a preferred embodiment, plants are transformed with *Agrobacterium*-derived vectors such as those described in Klee et al. (1987). Other well-known methods are available to insert the chimeric genes of the present invention into plant cells. Such alternative methods include biolistic approaches (described in Klein et al., 1987), electroporation, chemically-induced DNA uptake, and use of viruses or pollen as vectors.

When necessary for the transformation method, the chimeric genes of the present invention can be inserted into a plant transformation vector, e.g. the binary vector described by Bevan (1984). Plant transformation vectors can be derived by modifying the natural gene transfer system of *Agrobacterium tumefaciens*. The natural system comprises large Ti (tumor-inducing)-plasmids containing a large segment, known as T-DNA, which is transferred to transformed plants. Another segment of the Ti plasmid, the vir region, is responsible for T-DNA transfer. The T-DNA region is bordered by terminal repeats. In the modified binary vectors, the tumor inducing genes have been deleted and the functions of the vir region are utilized to transfer foreign DNA bordered by the T-DNA border sequences. The T-region also contains a selectable marker for antibiotic resistance, and a multiple cloning site for inserting sequences for transfer. Such engineered strains are known as "disarmed" *A. tumefaciens* strains, and allow the efficient transfer of sequences bordered by the T-region into the nuclear genome of plants.

Surface-sterilized leaf disks and other susceptible tissues are inoculated with the "disarmed" foreign DNA-containing *A. tumefaciens*, cultured for a number of days, and then transferred to antibiotic-containing medium. Transformed shoots are then selected after rooting in medium containing the appropriate antibiotic, and transferred to soil. Transgenic plants are pollinated and seeds from these plants are collected and grown on antibiotic medium.

Expression of a heterologous or reporter gene in developing seeds, young seedlings and mature plants can be monitored by immunological, histochemical or activity assays. As discussed herein, the choice of an assay for expression of the chimeric gene depends upon the nature of the heterologous coding region. For example, Northern analysis can be used to assess transcription if appropriate nucleotide probes are available. If antibodies to the polypeptide encoded by the heterologous gene are available, Western analysis and immunohistochemical localization can be used to assess the production and localization of the polypeptide. Depending upon the heterologous gene, appropriate biochemical assays can be used. For example, acetyltransferases are detected by measuring acetylation of a standard substrate. The expression of a lipid desaturase gene can be assayed by analysis of fatty acid methyl esters (FAMES).

Another aspect of the present invention provides transgenic plants or progeny of these plants containing the chimeric genes of the invention. Both monocotyledonous and dicotyledonous plants are contemplated. Plant cells are transformed with the chimeric genes by any of the plant

transformation methods described above. The transformed plant cell, usually in the form of a callus culture, leaf disk, explant or whole plant (via the vacuum infiltration method of Bechtold et al., 1993) is regenerated into a complete transgenic plant by methods well-known to one of ordinary skill in the art (e.g., Horsh et al., 1985). In a preferred embodiment, the transgenic plant is sunflower, cotton, oil seed rape, maize, tobacco, Arabidopsis, peanut or soybean. Since progeny of transformed plants inherit the chimeric genes, seeds or cuttings from transformed plants are used to maintain the transgenic line.

DEFINITIONS

The term "alpha globulin gene promoter and 5' untranslated region" ("AGP") as used herein refers to the 1144 bp DNA sequence shown in FIG. 1A (SEQ ID NO:1), any larger DNA sequence comprising this 1144 bp sequence, and any smaller DNA sequence which is comprised of part of the 1144 bp sequence and which functions transcriptionally in the same manner or in a similar manner as the full length 1144 bp sequence in terms of spatio-developmental expression patterns and/or expression level.

The term "alpha globulin gene promoter" as used herein refers to the 1108 bp DNA sequence shown in FIG. 1A (SEQ ID NO:2), any larger DNA sequence comprising this 1108 bp sequence, and any smaller DNA sequence which is comprised of part of the 1108 bp sequence and which functions transcriptionally in the same manner or in a similar manner as the full length 1108 bp sequence in terms of spatio-developmental expression patterns and/or expression level.

As used herein, the term "operatively linked" means that a regulatory region, such as a promoter, is connected to a coding region in such a way that the transcription of that coding region is controlled and regulated by that regulatory region. Methods for operatively linking a promoter to a coding region are well known in the art.

As used herein, the term "cassette" refers to a nucleotide sequence capable of expressing a particular gene if said gene is inserted so as to be operably linked to one or more regulatory regions present in the nucleotide sequence. Thus, for example, the expression cassette may comprise a heterologous coding sequence which is desired to be expressed in a plant seed. The expression cassettes and expression vectors of the present invention are therefore useful for directing seed-specific expression of any number of heterologous genes. The term "seed-specific expression" as used herein, refers to expression in the embryo portion of a plant seed.

EXAMPLES

FIG. 1 shows the α-globulin promoter sequence and reporter gene construct. FIG. 1A (SEQ ID NO. 1) shows the 1144 bp sequence that was isolated and functionally characterized in this study. Putative *cis*-acting elements are shown inside boxes. The transcription initiation site is indicated with +1. The 5' untranslated region is shown in italics and the additional, unpublished 336 bp sequence is underlined. The 336 bp sequence is shown in FIG. 10 (SEQ ID NO. 3). Following the

method described by Siebert et al. (1995), the 5' flanking promoter region was cloned using the sequence information from an α-globulin clone from a cotton staged-embryo cDNA library. The 772 bp of the clone toward the 3' end of the sequence presented here matched that of the published, 5' genomic flanking sequence for α-globulin gene B (Chlan et al., 1987). Further PCR walks resulted in an additional 336 nucleotides of upstream sequence (underlined). Based on the promoter sequence information, primers were designed to amplify an 1144 nucleotide long fragment containing the combined promoter and the untranslated leader region of the α-globulin B gene from cotton (cv. Coker 312) genomic DNA. The primers used were: AGP5= 5'-aag-ctt-gca-tgc-ctg-cag-CTA-TTT-TCA-TCC-TAT-TTA-GAA-ATC-3'; AGP3= 5'-ggg-acg-cgt-atc-GAT-TAC-GAT-AAG-CTC-TGT-ATT-TTG-3' (unique restriction sites incorporated into the primers are indicated in lowercase). The amplified PCR product was cloned into the TA cloning vector, pCRII (Invitrogen) resulting in pCRII-AGP. The integrity of the insert was verified by sequencing. Amplification from genomic cotton DNA with the described primers followed by routine cloning and sequencing allows anyone of skill in the art to acquire this DNA fragment without undue experimentation.

The α-globulin promoter from pCRII-AGP was then introduced as a *Hin*dIII-*Xba*I fragment into the polylinker sequence located upstream of the *gus*A gene in pBI101.3 (Clontech). An out-of-frame ATG (from pCRII polylinker) found upsteam of the GUS coding sequence was removed by deleting the region between the *Not*I and *Sma*I sites to create the test construct pBIAGPGUS. The entire putative promoter and 5' UTR were sequenced to verify the integrity of the final construct. The binary vector, pBIAGPGUS, shown in FIG. 1b, which harbors *npt*II as the plant selectable marker gene, was then introduced into *Agrobacterium* strains LBA4404 and GV3101 using the method described by An et al. (1988).

Referring to FIG. 1A, the TATA box and CAAT box are shown in bold letters and the 5' untranslated region is shown in italics. Visual analysis of the promoter sequence revealed a number of putative DNA motifs that may be involved in tissue-specific transcriptional regulation of the α-globulin gene B. There are four CANNTG motifs (Kawagoe and Murai, 1992), one CATGCACA (RY repeat, Dickinson et al., 1988), and two AACACA motifs (Goldberg, 1986). These *cis*-elements are believed to confer seed-specific expression to the promoter. Transient expression assays suggest a high degree of tissue (seed)-specificity for the 1108 bp sequence shown in FIG. 11 (SEQ NO. ID: 2). Functional analyses of this sequence were performed by stable transformation of three different species with a binary vector construct, shown in FIG. 1B, containing the reporter gene, β-glucuronidase, under the control of the α-globulin promoter.

Histochemical localization of GUS activity during seed development. Expression of β-glucuronidase gene, under the control of α-globulin promoter, was first tested using transient expression assays following particle bombardment-mediated transformation of developing embryos,

endosperm and leaves of sorghum and cotton. The results (not shown) indicated that the promoter was active only in developing embryos of cotton. On the basis of these results, stable transformations of tobacco, *Arabidopsis*, and cotton were performed for more detailed characterization of the cotton α-globulin promoter activity.

GUS assays were performed on T1 seeds of tobacco and cotton and T2 seeds of *Arabidopsis* as described by Jefferson et al. (1987). These generations of seeds will segregate for transgenes. In order to minimize the variation introduced by null segregants and homozygous seeds, the assay was performed with a large number of seeds. Assays were carried out as triplicates for each transgenic line with 25 (~2 gm), 150 (~15 mg), and 300 (~5 mg) seeds in each replicate for cotton, tobacco and *Arabidopsis,* respectively. Total protein was measured by using the method of Bradford (1976). GUS activity was normalized to the total protein and the results are presented as GUS specific activity (nanomole of 4-MU released per mg protein per min). GUS expression analyses were also carried out in leaf, root, stem and floral tissues of a plant that showed GUS activity in its seeds.

The seeds of T1 homozygous tobacco and T2 homozygous *Arabidopsis* were germinated on MSO medium and the seedlings at different days after germination were histochemically assayed (in the case of *Arabidopsis*) or fluorometrically assayed (in the case of tobacco) for GUS activity. After GUS staining, the seedlings were treated in ethanol to clear chlorophyll. In case of tobacco and *Arabidopsis*, the embryos/seedlings were photographed using Kodak Elitechrome Tungsten 160T film. The slides were then scanned and digitally enlarged. The cotton embryo images were captured using a Zeiss AxioCam digital camera coupled to a Zeiss M^2BIO Zoom Stereo/Compound microscope. FIG. 2 was compiled using Canvas 7.0 software.

Seeds from a T0 transgenic cotton plant showing seed-specific GUS expression were first germinated on 100 mg/l kanamycin to eliminate null segregants. Those seeds that germinated and grew in the presence of kanamycin were transferred to soil and grown to maturity. The zygosity status of these T1 plants was determined by GUS histochemical analysis on the seeds. One homozygous plant and one hemizygous plant were selected for quantitative analysis of GUS activity in their seeds. Embryos isolated from the seeds were analyzed individually for GUS activity using the fluorometric procedure described earlier.

FIG. 2 shows the histochemical localization of GUS activity in developing embryos from stably transformed tobacco, cotton, and *Arabidopsis* plants and in germinating *Arabidopsis* seedlings. Histochemical analysis of GUS activity was useful in identifying the timing and localization of α-globulin promoter-regulated expression. Histochemical analysis results for GUS activity in the embryos isolated from seeds at various stages of development in embryos from T1-homozygous tobacco plant are shown in FIGS. 2A-E. AGP:*gus*A expression was evaluated in the seeds from three T1-homozygous tobacco plants. *Nicotiana tabacum* cv. Havana was transformed with

Agrobacterium strain LBA4404(pBIAGPGUS) using the leaf disc transformation method (Horsch et al., 1988). Transformants were selected on regeneration medium (MS salts, 100 mg/l myo-inositol, 0.4 mg/l thiamine HCl, 4 μM BAP, 0.5 μM NAA, 3% sucrose, pH 5.6, solidified with 0.8% Difco-Bacto agar) containing 100 mg/l kanamycin and 500 mg/l carbenicillin. Regenerated shoots were excised and grown on MSO medium (MS salts, B-5 organics, 2% sucrose, pH 5.7, solidified with 0.8% Difco-Bacto agar) containing 100 mg/l kanamycin and 500 mg/l carbenicillin. Plants with good root systems were transferred to soil and grown to maturity in the greenhouse. Isolation of embryos from seeds of several capsules and their microscopic visualization indicated that embryos reached the heart stage around 9 days post anthesis (dpa). No visible GUS activity was detected in embryos at heart or late heart stages. However, GUS activity was observed in embryos at late torpedo and older stages of development.

FIGS. 2F-2N show GUS activity in embryos from T1-homozygous cotton plant. Histochemical analysis of GUS activity was carried out in developing embryos isolated from the seeds of a T1-homozygous cotton plant. The hypocotyl segments of cotton (*Gossypium hirsutum* cv. Coker 312) seedlings were transformed with *Agrobacterium* [LBA4404(pBIAGPGUS)] by following the method described by Sunilkumar and Rathore (2001). Plants were regenerated from kanamycin-resistant transgenic calli and grown to maturity. GUS staining was first detected in embryos at 16 dpa. At this stage, as shown in FIGS. 2F and 2G, the cotyledons had just begun to expand and GUS activity appeared just below the cotyledons, at the junction of cotyledons and hypocotyl. The activity increased and spread throughout the embryo as the seed development progressed as shown in FIGS. 2H-2N. Intense staining was observed in embryos 40 dpa and in mature embryos isolated from dry seeds. FIG. 2O shows an embryo isolated from a null segregant seed that was negative following histochemical GUS assay. Results from histochemical localization of GUS activity in mature embryos from transgenic plants of three plant species suggests that the 1108 bp promoter region has the required *cis*-acting domains that confer expression in the embryo.

FIGS. 2P-2T show GUS activity in developing embryos from the seeds of T2-homozygous *Arabidopsis thaliana* C24 plant. Seeds from a homozygous T2 generation of *Arabidopsis* transformed with AGP:*gus*A were used for histochemical analyses. *Arabidopsis thaliana* C24 plants were transformed by the vacuum infiltration method (Bechtold and Pelletier, 1998) using the *Agrobacterium* strain GV3101 (pBIAGPGUS). Transformed seeds (T1) were selected on MSO medium containing 50 mg/l kanamycin. The kanamycin-resistant plants were transferred to soil and grown to maturity in a growth room (23°C, 65% humidity, 14h/10h photoperiod).

Expression of AGP:*gus*A was monitored in the embryos isolated from seeds at various developmental stages. GUS staining was not visible in heart stage and late heart stage embryos (results not shown). A low level of GUS activity was observed in the torpedo stage embryos, shown

in FIG. 2P, and the intensity of blue staining progressively increased as the embryos grew to maturity as shown in FIGS 2Q-2T. An intense GUS staining was found in the embryos isolated from dry seeds. Taken together, results from these three dicot species suggest that gene expression driven by AGP is confined to middle to late stages of embryo development.

FIGS. 2U-2Y show GUS activity during seed germination in transgenic *Arabidopsis*. In order to determine if AGP activity is confined strictly to developing embryos/seeds, GUS activity was monitored in germinating *Arabidopsis* seedlings. GUS activity was analyzed in germinating seedlings using the histochemical method. Results presented in FIGS. 2U-Y show that the intensity of GUS staining decreased progressively as the seedlings grew. At 5 days post imbibition, there was still some residual GUS activity visible. However, after 7 days, faint patches of blue staining were observed only at the two ends of the hypocotyl. GUS staining was not visible in cotyledons, root or in the middle portion of the hypocotyl. No GUS activity was observed in seedlings beyond 7 days post imbibition (FIG. 2Y).

FIG. 3 shows the developmental regulation of GUS expression by the α-globulin promoter in tobacco via quantitative analysis. Histochemical analysis does not permit detection of low levels of GUS activity and also does not give a precise measure of increase in the level of GUS expression. Therefore, AGP activity during seed development was studied by monitoring GUS expression in developing seeds (tobacco) and developing embryos (cotton) by quantitative, fluorometric GUS assay at various time points after flowering. As shown in Fig. 3A, measurable GUS-specific activity was first detected at 12 dpa in the seeds from a T1 homozygous tobacco plant. The activity then increased rapidly, finally reaching a maximum at 20 dpa.

FIG. 3B shows GUS activity in tobacco seedlings during germination at different days post imbibition. Surface sterilized seeds from a T1 homozygous tobacco plant were germinated on MS medium (Murashige and Skoog, 1962). GUS fluorometric assay was carried out using the extracts from whole seedlings at 0, 2, 4, 6, 8 and 10 days post imbibition. GUS activity decreased continuously following seed germination (FIG. 3B) and only 2% of the initial activity was found after 8 days. No GUS activity was detected in seedlings 10 days after germination. The fact that GUS activity rapidly drops to undetectable levels following seed germination in both *Arabidopsis* (Figs. 2A-2E) and tobacco (Fig. 3B) suggests that the promoter is active only during seed development and inactive during seed germination and in mature plant.

FIG. 4 shows the developmental regulation of GUS expression by the α-globulin promoter in cotton embryos via quantitative analysis. Because of large seed size, the relatively slow process of embryo development, and the ease with which the embryos can be isolated from developing seeds, cotton offered the best system to carry out detailed characterization of AGP:*gus*A expression at the single seed level. The results from quantitative analysis for GUS activity, protein levels and fresh

weight in developing embryos, isolated from the seeds from a T1 homozygous cotton plant, are shown in FIG. 4. This plant was grown in the greenhouse in the month of April and at this time of the year, the bolls opened at about 43 dpa. GUS expression was first detected at 15 dpa (60 pmoles/mg protein/min). Thereafter, there was a slow increase in GUS activity till 20 dpa, followed by a rapid rise until 40 dpa. From this peak until seed maturity, there was a small but statistically significant decline in activity. During seed development, the protein levels (as measured in the GUS extraction buffer) increased rapidly from 15 dpa to 30 dpa, followed by a slow increase till 40 dpa before leveling off. It was not possible to accurately weigh the embryos before 18 dpa. However, from this point on, embryo fresh weight increased until 40 dpa, followed by a decrease as the seed reached dry state. These results confirm the histochemical analysis. The AGP:*gus*A expression begins in cotton embryos at around 15 dpa, and the activity either levels off or declines beyond 40 dpa.

Table 1 shows GUS specific activity in various tissues of a T1-homozygous transgenic cotton plant and in control seeds. Histochemical GUS analysis was performed on various parts of three different T0 transgenic cotton plants that expressed the reporter gene in the embryos. GUS activity-dependent histochemical staining was not detected in tissues such as stem, leaf, petiole, flower stock, sepals, petals or square of the transgenic plant. In addition, more sensitive fluorometric analyses were performed to detect AGP activity in different organs and tissues of one of the transgenic cotton plant. Results from this analysis, presented in Table 1, show clearly that no measurable GUS activity was present in stem, leaf, floral bud, pollen, and root. A high level of GUS activity was detected only in the seeds. These results suggest that AGP-driven transgene activity is tightly controlled and is specific to the seed.

Table 1. GUS specific activity in various tissues of a T1-homozygous transgenic cotton plant and in control seeds.

Tissue type	GUS activity [a] (nmole 4-MU/ mg protein/ min)
Stem	0.018 ± 0.002
Leaf	0.014 ± 0.005
Root	0.12 ± 0.006
Floral bud	0.11 ± 0.05
Pollen	0.024[b]
Transgenic seed[c]	349.9 ± 55
Control seed[c]	0.002 ± 0.0004

[a]Values are mean GUS activity ± SE from three replicates. [b]The number of replicates were not sufficient to calculate SE (5.7 mg pollen was used in the assay). [c]Assay was performed in embryos collected from 10 seeds for each replicate.

FIG. 5 shows GUS activity, plotted on a log scale, in seeds from independent transgenic lines of T0 tobacco, T1 *Arabidopsis*, and T0 cotton. Preliminary results had indicated that AGP-driven GUS activity differed greatly amongst these three species. Extensive analyses were performed on seeds from a number of independent transgenic lines (that were positive for GUS activity as tested by histochemical method) from *Arabidopsis*, tobacco and cotton to confirm this observation. As shown in FIG. 5, GUS activity in the seeds from 11 independent transgenic tobacco lines ranged from 0.6 to 18 nanomole 4-MU/mg protein/min. Similar analysis in seeds from 10 independent transgenic *Arabidopsis* lines showed a range of 49 to 203 nanomole 4-MU/mg protein/min. GUS activity in 10 independent transgenic cotton lines ranged from 118 to 1777 nanomole 4-MU/mg protein/min. Similar high levels of seed-specific promoter expression has been reported also in maize seeds obtained from glutelin promoter:*gus*A and zein promoter:*gus*A transformants (Russell and Fromm, 1997). The results suggest that the cotton AGP, although recognized in different heterologous systems as a seed-specific promoter, showed the highest level of activity in cotton.

FIG. 6 shows GUS specific activity in individual embryos isolated from seeds from a T1 homozygous and seeds from a single T1 hemizygous cotton plant. Both homozygous and hemizygous T1 pants were derived from a single T0 transgenic line. The large seed size of cotton allowed analysis of GUS activity at a single seed level. This provided us with an opportunity to get a quantitative measure of GUS activity in individual seeds within the segregating T2 seed population produced by a hemizygous T1 plant and compare these values with activities in individual seeds produced by a homozygous T1 plant. As shown in FIG. 6, all of the T2 seeds from the homozygous T1 parent showed GUS activity (FIG. 6, bottom histograms) suggesting that reintroduction of a native promoter, even under homozygous condition, did not result in transgene silencing in this line. T2 seeds from the hemizygous T1 parent showed clear phenotypic segregation (3:1) for the transgene activity (FIG. 6, top histograms). Moreover, among the seeds showing GUS activity, two different levels of activity was apparent in majority of the cases. The higher level activity in about one fourth of T2 seeds from the hemizygous parent was similar to the level seen in the T2 seeds from the homozygous parent. Thus, the two different levels of GUS activity in the seeds from the hemizygous plant may be a result of either hemizygous or homozygous transgenic status of the individual seed suggesting a gene dose effect.

FIG. 7 shows that the α-globulin promoter drives antisense expression of the δ-12 desaturase gene from cotton to increase the levels of oleic acid in different transgenic lines of cottonseeds. Cotton (Coker 312) was transformed with a construct where a δ-12 desaturase gene from *Gossypium hirsutum*, in antisense orientation was under the control of cotton α-globulin promoter. Cotton transformation was performed as described by Sunilkumar and Rathore (2001). A total of 45 plants were regenerated from 26 independent transgenic callus lines. The kernels from single T1 cotton

seeds or a pooled sample of randomly-picked 30 seeds from each plant were homogenized to a fine powder using agate mortar and pestle. Total fatty acids was extracted from a sample of 50 mg of this powder as described by Dahmer et al. (1989). Fatty acid analysis was performed using a gas chromatograph. The results are expressed as percentage of total fatty acids. The fatty acid composition of cottonseed oil is: myristic acid (0.9%), palmitic acid (24.7%), stearic acid (2.3%), oleic acid (17.6%), and linoleic acid (53.3%) (White et al., 2000). The levels of oleic acid in the T1 seeds from transgenic plants ranged from 15% to 29% (FIG. 7). Linoleic acid levels ranged between 53% to 40% in

these plants. The lines with decreased levels of linoleic acid showed a concomitant increase in oleic acid levels. This negative correlation is to be expected if the alteration is a result of suppression of δ-12 desaturase activity.

FIG. 8 shows the fatty acid levels of four lines of high-oleate cotton seeds at the individual seed level. Four high-oleate lines were chosen for fatty acid analysis at individual seed level. Since T1 seeds will be segregating for the transgene, a few seeds (null segregants) are expected to have wild type levels of oleic acid/linoleic acid. However, majority of the seeds will exhibit higher oleic acid / lower linoleic acid phenotype. As shown in FIG. 8, some seeds from the transgenic lines H50-2 and H41-1 had oleic acid levels as high as 34.2% and 34.3%, respectively. As expected, few seeds (probably null segregants) from all the four lines exhibited wild-type levels of oleic acid/linoleic acid.

FIG. 9 shows the fatty acid levels in T2 cotton seeds. Seeds were germinated from two of the high-oleate lines and the plants were grown to maturity. Fatty acid analysis was performed on a pooled sample of randomly picked 30 T2 seeds. T2 seeds from Plant #13 and #15 from line H50-2 had oleic acid content of 32.3% and 32.8%, respectively (FIG. 9). Their linoleic acid contents were 35.9% and 36.7%, respectively. In line H41-1, T2 seeds from Plant #1 contained 32% oleic acid and 37% linoleic acid.

As shown in FIG. 9, there was over 80% increase in the levels of oleic acid in the seeds from some of the transgenic lines compared to the wild-type controls. This increase in the oleic acid was associated with a concomitant reduction of approximately 30% in the levels of linoleic acid. As reflected in FIGS. 7-9, the α-globulin promoter effectively manipulates fatty acid levels in oil seeds by driving expression of gene silencing constructs.

Table 2 shows GUS activity in various tissues from control and AGP:*gusA* transgenic cotton plants that reflects stringent, seed-specific expression of the of α-globulin promoter. Tight regulation of seed-specific promoter expression is important in cases where even a minimal level of promoter activity in the vegetative parts is not acceptable. To determine whether the α-globulin promoter is active exclusively in the seeds and whether the cotton α-globulin promoter can be used to express the

transgenes in seeds whose expression in non-seed tissues is undesirable, GUS activity in various tissues from control and AGP:*gusA* transgenic plants was measured. As shown in Table 2, GUS assays, based on the quantitation of MU (a fluorescent reaction product), show that AGP:*gusA* gene was expressed only in the seeds and very low levels of fluorescence readings were noted for vegetative and floral tissues.

Table 2. GUS activity in various tissues from control and AGP:*gusA* transgenic cotton plants

Tissue	GUS activity nmole 4-MU/ mg protein /min	
	Control	AGP:*gusA*
Stem	0.06 ± 0.008	0.018 ± 0.002
Root	0.139 ± 0.005	0.12 ± 0.006
Pollen	0.018 ± 0.0	0.024 *
Flower bud	0.801 ± 0.025	0.11 ± 0.05
Leaf	0.037 ± 0.002	0.014 ± 0.005
Seed	0.002 ± 0.0004	349.9 ± 55

* Number of replicates were not sufficient to calculate SE.

Assays were done in triplicates. Numbers are Mean ± Std Error.

Tables 3A and 3B show GUS activity in water-stressed AGP:*gusA* transgenic plants. Some members of *Lea* class seed-specific promoters are known to be activated in vegetative tissues by ABA as well as drought conditions (Seffens et al., 1990; Vivekananda et al., 1992, Siddiqui et al., 1998). Exogenous ABA was shown to induce β-phaseolin promoter driving *gusA* gene in isolated embryos of transgenic tobacco (Bustos et al., 1998). To rule out the possibility that the cotton α-globulin promoter (AGP) may be activated in vegetative parts under water-stress condition that are known to result in increased levels of endogenous ABA, GUS fluorometric assays were performed on leaf extracts of plants from three different transgenic lines that were subjected to water stress by withholding watering. Leaf samples were analyzed for GUS activity at different time points following the last watering until the time they showed complete wilting. As shown in Tables 3A and 3B, no measurable GUS activity was detected in any of the leaf samples from these three transgenic plants even after they were completely wilted. The results show that α-globulin promoter is active exclusively in the seeds and it is not induced in the vegetative parts of the plant by water stress conditions.

Table 3A. GUS activity in water stressed AGP:*gus*A transgenic plants #1 and #2. Assays were performed on leaf extracts.

Plant #	Days after last watering (nmole 4-MU/ mg protein /min*)						
	2 days	3 days	5 days	7 days	8 days	9 days	14 days
1	0.012 ± 0.0043	0.016 ± 0.0046	0.0071 ± 0.0027	0.014 ± 0.0024	0.022 ± 0.0041	0.015± 0.0034	0.01 ± 0.00089
2	0.011± 0.0039	0.017± 0.0057	0.0083 ± 0.0036	0.01 ± 0.0039	0.014 ± 0.0041	0.02 ± 0.0034	0.001 ± 0.00091

Table 3B. GUS activity in water stressed AGP:*gusA* transgenic plant #3. Assays were performed on leaf extracts.

Plant #	Days after last watering (nmole 4-MU/ mg protein /min*)						
	2 days	6 days	8 days	10 days	11 days	12 days	17 days
3	0.014 ± 0.006	0.014 ± 0.0073	0.009 ± 0.0022	0.012 ± 0.0033	0.018 ± 0.0054	0.019 ± 0.01	0.0013 ± 0.001

*Assays were done in triplicates. Numbers are Mean ± Std Error.

It is clear that the 1108 bp α-globulin promoter sequence from cotton, characterized in this study, can confer a strong seed-specific expression in cottonseed as well as in the seeds of two other dicots. AGP will be useful for any application involving transgene-mediated over-expression or suppression during seed development in dicots, thus adding to the availability of seed-specific promoters.

Various basics of the invention have been explained herein. The various techniques and devices disclosed represent a portion of that which those skilled in the art would readily understand from the teachings of this application. Details for the implementation thereof can be added by those with ordinary skill in the art. The accompanying figures may contain additional information not specifically discussed in the text and such information may be described without adding new subject matter. Additionally, various combinations and permutations of all elements or applications can be created and presented. All can be done to optimize performance in a specific application.

The various steps described herein can be combined with other steps, can occur in a variety of sequences unless otherwise specifically limited, various steps can be interlineated with the stated steps, and the stated steps can be split into multiple steps. Unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", should be understood to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps.

Further, any references mentioned in the application for this patent as well as all references listed in any list of references filed with the application are hereby incorporated by reference. However, to the extent statements might be considered inconsistent with the patenting of this invention such statements are expressly not to be considered as made by the applicant(s).

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CLAIMS

1. An isolated nucleic acid corresponding to an AG 5' regulatory region which directs seed-specific expression comprising the nucleotide sequence set forth in SEQ ID NO:1.
2. A plant transformation vector which comprises at least one nucleic acid of claim 1.
3. A plant cell comprising a nucleic acid of claim 1, said nucleic acid being heterologous to said plant cell.
4. A plant, or progeny of said plant, which has been regenerated from the plant cell of claim 3.
5. A transgenic plant, or progeny of said plant, comprising a nucleic acid of claim 1.
6. The plant of claim 4 wherein said plant is a cotton, tobacco, oil seed rape, maize or soybean plant.
7. The plant of claim 6 wherein said plant is a cotton, tobacco, oil seed rape, maize or soybean plant.
8. An expression cassette which comprises at least one AG 5' regulatory region of claim 1 operably linked to at least one a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.
9. The expression cassette of claim 8 wherein the heterologous gene is at least one of a fatty acid synthesis gene or a lipid metabolism gene.
10. The expression cassette of claim 9 wherein the heterologous gene is selected from the group consisting of an acetyl-coA carboxylase gene, a ketoacyl synthase gene, a malonyl transacylase gene, a lipid desaturase gene, an acyl carrier protein (ACP) gene, a thioesterase gene, an acetyl transacylase gene, and an elongase gene.
11. The expression cassette of claim 9 wherein the lipid desaturase gene is selected from the group consisting of a Δ6-desaturase gene, a Δ12-desaturase gene, and a Δ15-desaturase gene.
12. An expression vector which comprises the expression cassette of claim 8.
13. A cell comprising the expression cassette of any one of claim 8.
14. A cell comprising the expression vector of claim 12.
15. The cell of claim 13 wherein said cell is a bacterial cell or a plant cell.
16. The cell of claim 14 wherein said cell is a bacterial cell or a plant cell.
17. A transgenic plant comprising the expression cassette of claim 8.
18. A transgenic plant comprising the expression vector of claims 14.
19. A plant which has been regenerated from the plant cell of claim 15.
20. A plant which has been regenerated from the plant cell of claim 14.
21. The plant of claim 18 wherein said plant is a sunflower, soybean, maize, cotton, tobacco, peanut, oil seed rape or Arabidopisis plant.
22. The plant of claim 19 wherein said plant is a sunflower, soybean, maize, cotton, tobacco, peanut, oil seed rape or Arabidopisis plant.

23. Progeny of the plant of claim 17.
24. Progeny of the plant of claim 18.
25. Seed from the plant of claim 17.
26. Seed from the plant of claim 18.
27. An expression vector which comprises the expression cassette of claim 9.
28. A cell comprising the expression cassette of claim 9.
29. A transgenic plant comprising the expression cassette of claim 9.
30. An expression vector which comprises the expression cassette of claim 10.
31. A cell comprising the expression cassette of claim 10.
32. A transgenic plant comprising the expression cassette of claim 10.
33. An expression vector which comprises the expression cassette of claim 11.
34. A cell comprising the expression cassette of claim 11.
35. A transgenic plant comprising the expression cassette of claim 11.
36. An isolated nucleic acid corresponding to an AG regulatory region which directs seed specific expression comprising the nucleotide sequence set forth in SEQ ID NO:2.
37. A plant transformation vector which comprises at least one nucleic acid of claim 36.
38. A plant cell comprising a nucleic acid of claim 36, said nucleic acid being heterologous to said plant cell.
39. A plant, or progeny of said plant, which has been regenerated from the plant cell of claim 38.
40. A transgenic plant, or progeny of said plant, comprising a nucleic acid of claim 36.
41. The plant of claim 39 wherein said plant is a cotton, tobacco, oil seed rape, maize or soybean plant.
42. The plant of claim 40 wherein said plant is a cotton, tobacco, oil seed rape, maize or soybean plant.
43. An expression cassette which comprises at least one AGP 5' regulatory region of claim 36 operably linked to at least one of a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.
44. The expression cassette of claim 43 wherein the heterologous gene is at least one of a fatty acid synthesis gene or a lipid metabolism gene.
45. The expression cassette of claim 44 wherein the heterologous gene is selected from the group consisting of an acetyl-coA carboxylase gene, a ketoacyl synthase gene, a malonyl transacylase gene, a lipid desaturase gene, an acyl carrier protein (ACP) gene, a thioesterase gene, an acetyl transacylase gene, and an elongase gene.
46. The expression cassette of claim 44 wherein the lipid desaturase gene is selected from the group consisting of a Δ6-desaturase gene, a Δ12-desaturase gene, and a Δ15-desaturase gene.
47. An expression vector which comprises the expression cassette of claim 43.

48. A cell comprising the expression cassette of any one of claim 43.
49. A cell comprising the expression vector of claim 47.
50. The cell of claim 48 wherein said cell is a bacterial cell or a plant cell.
51. The cell of claim 49 wherein said cell is a bacterial cell or a plant cell.
52. A transgenic plant comprising the expression cassette of claim 43.
53. A transgenic plant comprising the expression vector of claims 49.
54. A plant which has been regenerated from the plant cell of claim 50.
55. A plant which has been regenerated from the plant cell of claim 49.
56. The plant of claim 53 wherein said plant is a sunflower, soybean, maize, cotton, tobacco, peanut, oil seed rape or Arabidopisis plant.
57. The plant of claim 54 wherein said plant is a sunflower, soybean, maize, cotton, tobacco, peanut, oil seed rape or Arabidopisis plant.
58. Progeny of the plant of claim 52.
59. Progeny of the plant of claim 53.
60. Seed from the plant of claim 52.
61. Seed from the plant of claim 53.
62. An expression vector which comprises the expression cassette of claim 44.
63. A cell comprising the expression cassette of claim 44.
64. A transgenic plant comprising the expression cassette of claim 44.
65. An expression vector which comprises the expression cassette of claim 45.
66. A cell comprising the expression cassette of claim 45.
67. A transgenic plant comprising the expression cassette of claim 45.
68. An expression vector which comprises the expression cassette of claim 46.
69. A cell comprising the expression cassette of claim 46.
70. A transgenic plant comprising the expression cassette of claim 46.
71. A method for obtaining a plant which produces at least one seed having a protein content different from a plant of the same species not treated by this method, said method comprising:

a) transforming a host plant cell with a DNA construct, wherein said construct comprises, as operably linked components, an alpha globulin gene regulatory region, and a DNA sequence encoding a protein, wherein said components are functional in a plant cell, whereby said DNA construct becomes integrated into a genome of said plant cell;

b) regenerating a plant from said transformed plant cell; and

c) growing said plant under conditions whereby said DNA sequence of interest is expressed and a seed having said protein content is obtained.

72. The method of claim 71 wherein said alpha globulin gene regulatory region comprises that DNA sequence set forth in SEQ ID NO: 1.

73. The method of claim 71 wherein said alpha globulin gene regulatory region comprises that DNA sequence set forth in SEQ ID NO: 2.

74. The method of claim 71 wherein said alpha globulin gene regulatory region comprises that DNA sequence set forth in SEQ ID NO: 3.

75. The method of claim 71 wherein said DNA sequence encoding a protein is a heterologous gene.

76. The method of claims 72, 73, or 74 wherein said DNA sequence encodes a protein that alters the fatty acid content of said at least one seed.

77. A method for obtaining a plant which produces at least one seed having a protein content different from a plant of the same species not treated by this method, said method comprising:

transforming a host plant cell with a DNA construct, wherein said construct comprises, as operably linked components, an alpha globulin gene regulatory region, and a DNA sequence encoding a protein, wherein said components are functional in a plant cell.

78. A method for obtaining a plant which produces at least one seed having a protein content different from a plant of the same species not treated by this method, said method comprising:

transforming a host plant cell with a RNA or DNA, wherein said construct comprises, as operably linked components, an alpha globulin gene regulatory region, and an RNA or DNA sequence encoding a protein, wherein said components are functional in a plant cell.

79. A method for altering expression of a protein in a cell of a plant which produces at least one seed compared to a cell of a plant of the same species not treated by this method, said method comprising:

transferring an RNA construct to a host plant cell, wherein said construct comprises, as operably linked components, an alpha globulin gene regulatory region, and an RNA sequence that drives the transcription of an RNA that is complementary to an endogenous RNA encoding a protein, whereby expression of said endogenous protein is altered.

A

-1108 CTATTTTCAT CCTATTTAGA AATCCAAGTT GACACCTAAA ATTTAGTTGG ACTGCCATGT
-1048 AGGATTATCG TTAGAGAGAT AACGGAGCTT AACGGTAGAG TGATCACTTT GTAACAAAAT
-988 AATAACAAAA GTGACTAAAG TGTAACATTT CAAACATAAA TGATTAAAAT ATAACCTGAG
-928 GCAAACAAAA ATGACTATTT TTATAGATTA CCCTAAAATT AAAGAGTCAT GGCCCTAGCC
-868 CCTCGCCTAC TTGTTTGTTT TTAATAAACT AACATAGTAT AATATATTGT TAGGATTATA
-808 TAAAATTATT AATAAATAGT ATAATTAATT TAAAATTTAT GAAAAATAAA TTACCATATT
-748 TCTTAAATAC GTGGCACCTT ATGTTGGATT GGACTGTATA ACTTATATAC TATTATCTAT
-688 ATTGAATCCA AATCCTTACT TTTAAGCGTT TTTAGTGAAA CATTTTATTT TCCATTCTTA
-628 TTATATAAAT TTATATAATG ATATAATATG TAATACTTAG ATAATATTAT TGAAAAAGAA
-568 TAAAAATACC TCAAACTTTG AAAGGACTAA TTTGTATGAG CATCAAACGT ACAGGATACC
-508 AAAAGTATAC ATATCTGAAT TTGTTCATAT CTCCTGCAAC TCATAGATCA TCACCATGCA
-448 CAGCAACATG TGTACACTTG ACTTGTCCTC TATCAACTCA ACCCTTAACT CAGTGAATCG
-388 GGACATCTCT GTCTCACTTT AAAACCCTTC CCAGTTTCAA CACTCTTTGA ATTCAACTGA
-328 GTTCACATAC AACACAACAC AGTCCATCAT CTTTCTGCTG TTAAAGCATC ATCATTTCGC
-268 CCCTTCCAGT TACAGATGCA ACATGAACCC CCCTGCAACA AAGTTTGTCC GAACCTTGCT
-208 AGTACCATGT GAAGGGATGT GGCATCTCGA TATCTACCCA CCACTATACA AAAAAAAAAA
-148 AAAGAGACAA TATTTCGTCT TCTTTAATTT GCACACTCGT CATCTTGCAT GTCAATGTCT
-88 TCAACACGTT GATGAAGATT TGCATGCAAA AATATCACCT TCCACAGCTC CACCTTCTAT
+1
-28 AAATACATTA CCACTCTTTG CTATTACCAT CACACAGTAA CAAAATACAG AGCTTATCGT
+33 AATC

↳AT G of α-globulin B gene

B

FIG. 1

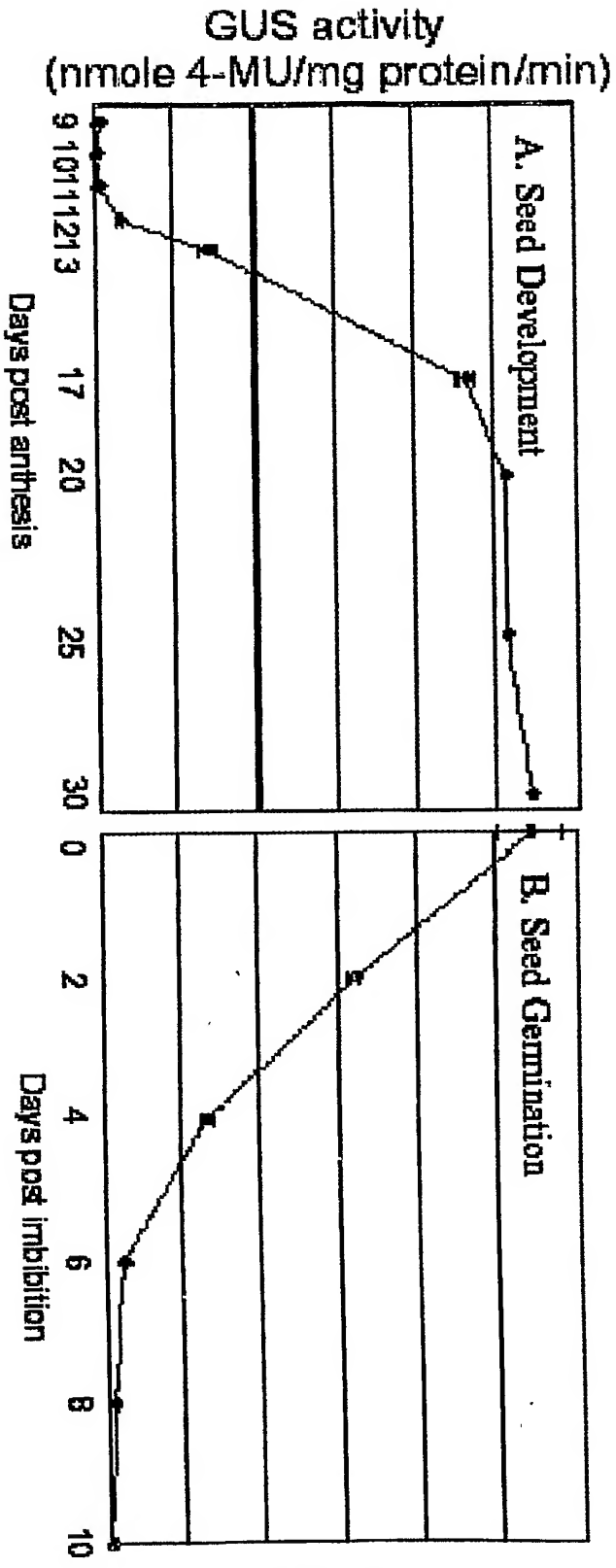
GUS activity
(nmole 4-MU/mg protein/min)
A. Seed Development
Days post anthesis
9 10 11 12 13 17 20 25 30
B. Seed Germination
Days post imbibition
0 2 4 6 8 10

FIG. 3

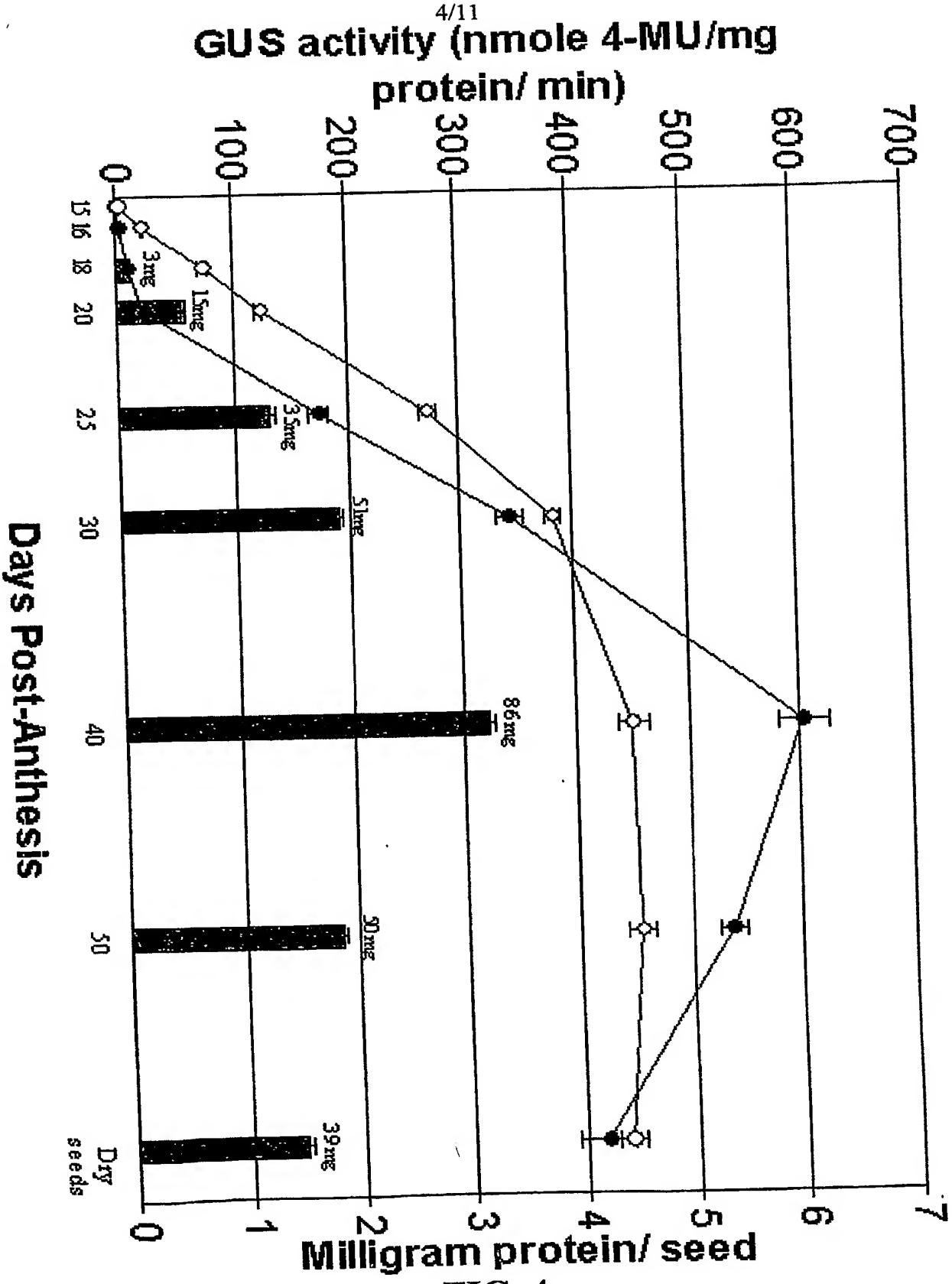
GUS activity (nmole 4-MU/mg protein/ min)
0
100
200
300
400
500
600
700
Days Post-Anthesis
15 16 18 20
25
30
40
50
Dry seeds
3mg
15mg
35mg
51mg
86mg
90mg
39mg
0
1
2
3
4
5
6
7
Milligram protein/ seed

FIG. 4

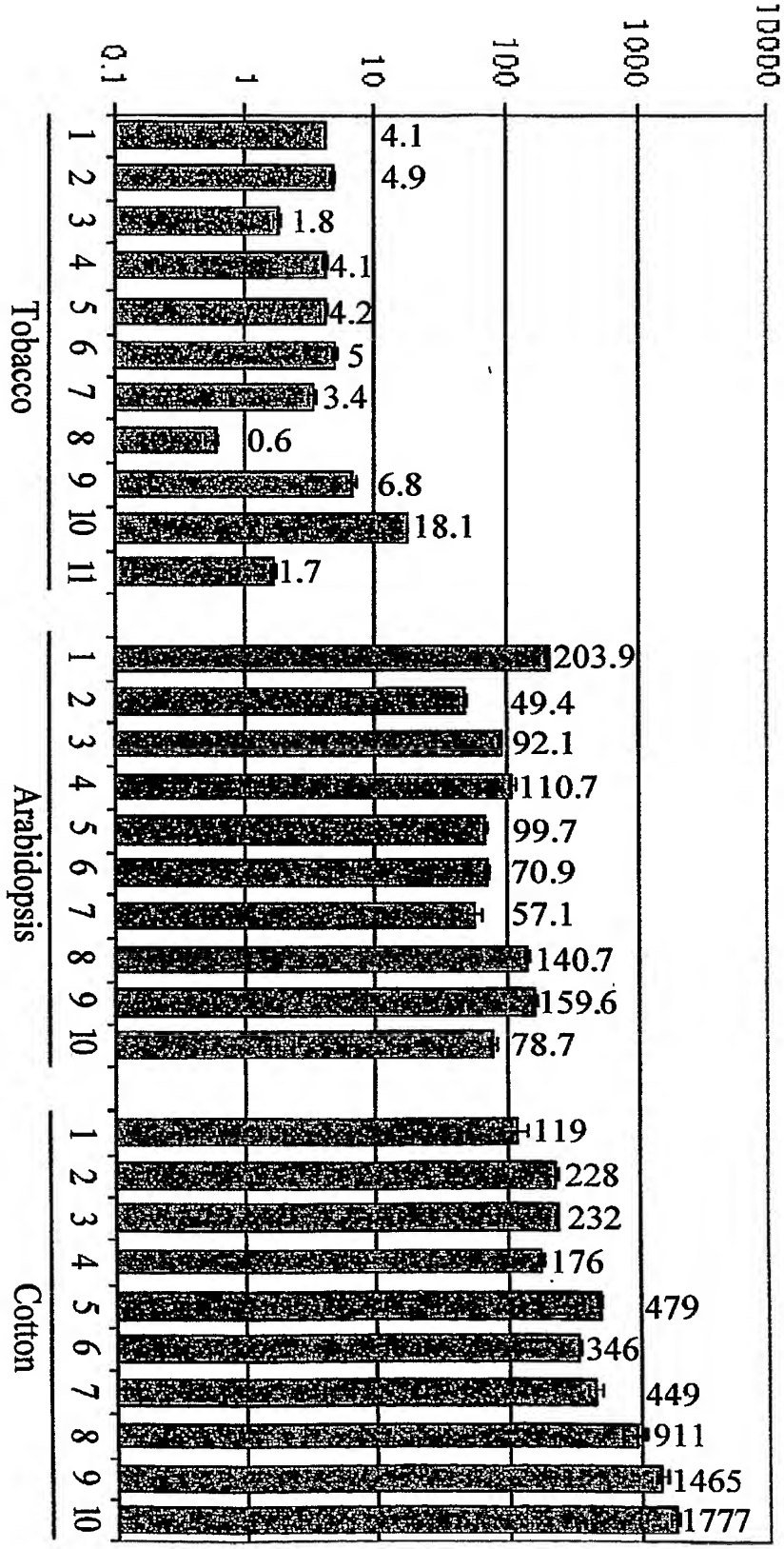
0.1
1
10
100
1000
10000
Tobacco
1 2 3 4 5 6 7 8 9 10 11
4.1
4.9
1.8
4.1
4.2
5
3.4
0.6
6.8
18.1
1.7
Arabidopsis
1 2 3 4 5 6 7 8 9 10
203.9
49.4
92.1
110.7
99.7
70.9
57.1
140.7
159.6
78.7
Cotton
1 2 3 4 5 6 7 8 9 10
119
228
232
176
479
346
449
911
1465
1777

FIG. 5

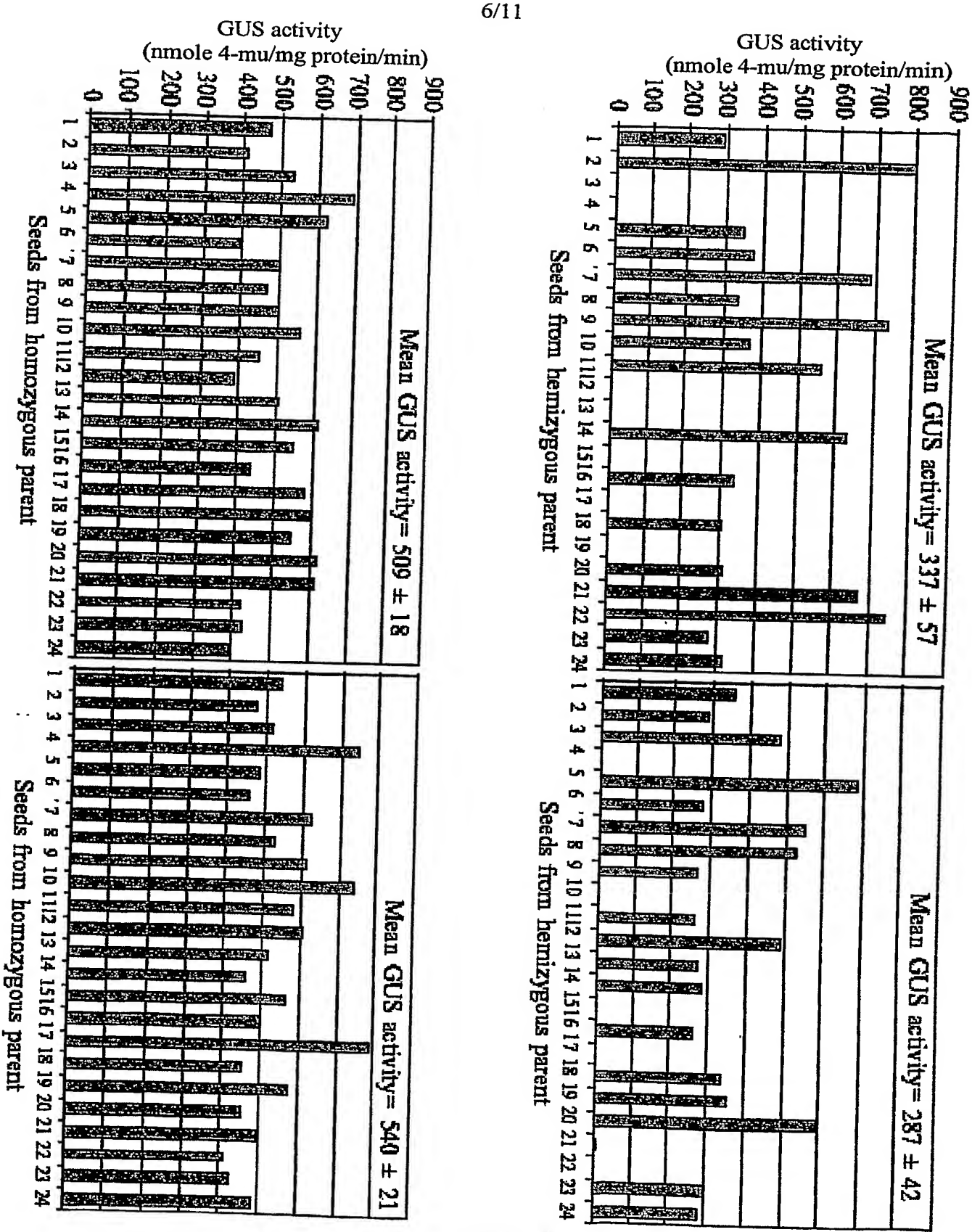
GUS activity
(nmole 4-mu/mg protein/min)
0
100
200
300
400
500
600
700
800
900
Mean GUS activity= 337 ± 57
Seeds from hemizygous parent
Mean GUS activity= 287 ± 42
Seeds from hemizygous parent
GUS activity
(nmole 4-mu/mg protein/min)
Mean GUS activity= 509 ± 18
Seeds from homozygous parent
Mean GUS activity= 540 ± 21
Seeds from homozygous parent

FIG. 6

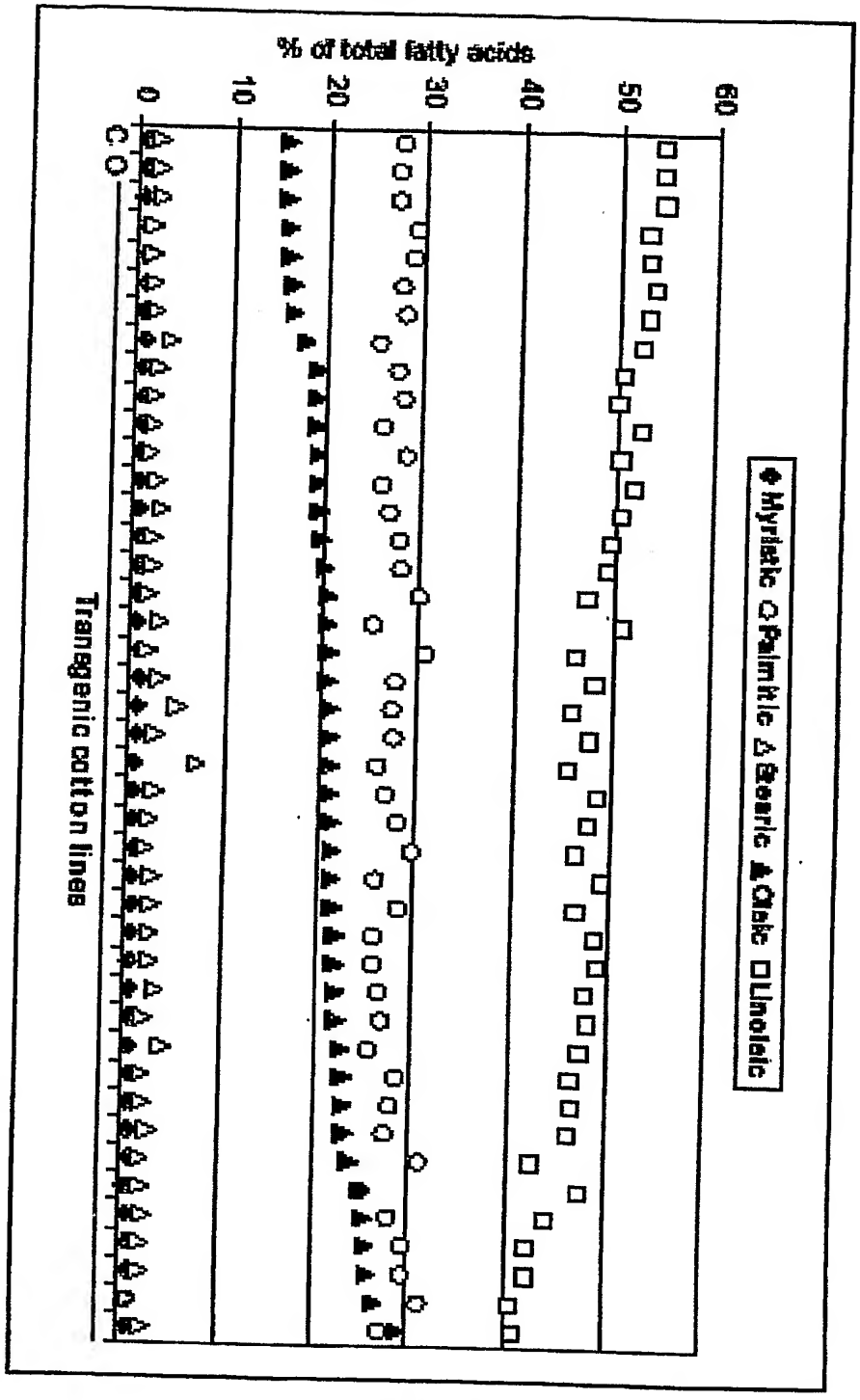
% of total fatty acids
0
10
20
30
40
50
60
Transgenic cotton lines
Myristic
Palmitic
Stearic
Oleic
Linoleic

FIG. 7

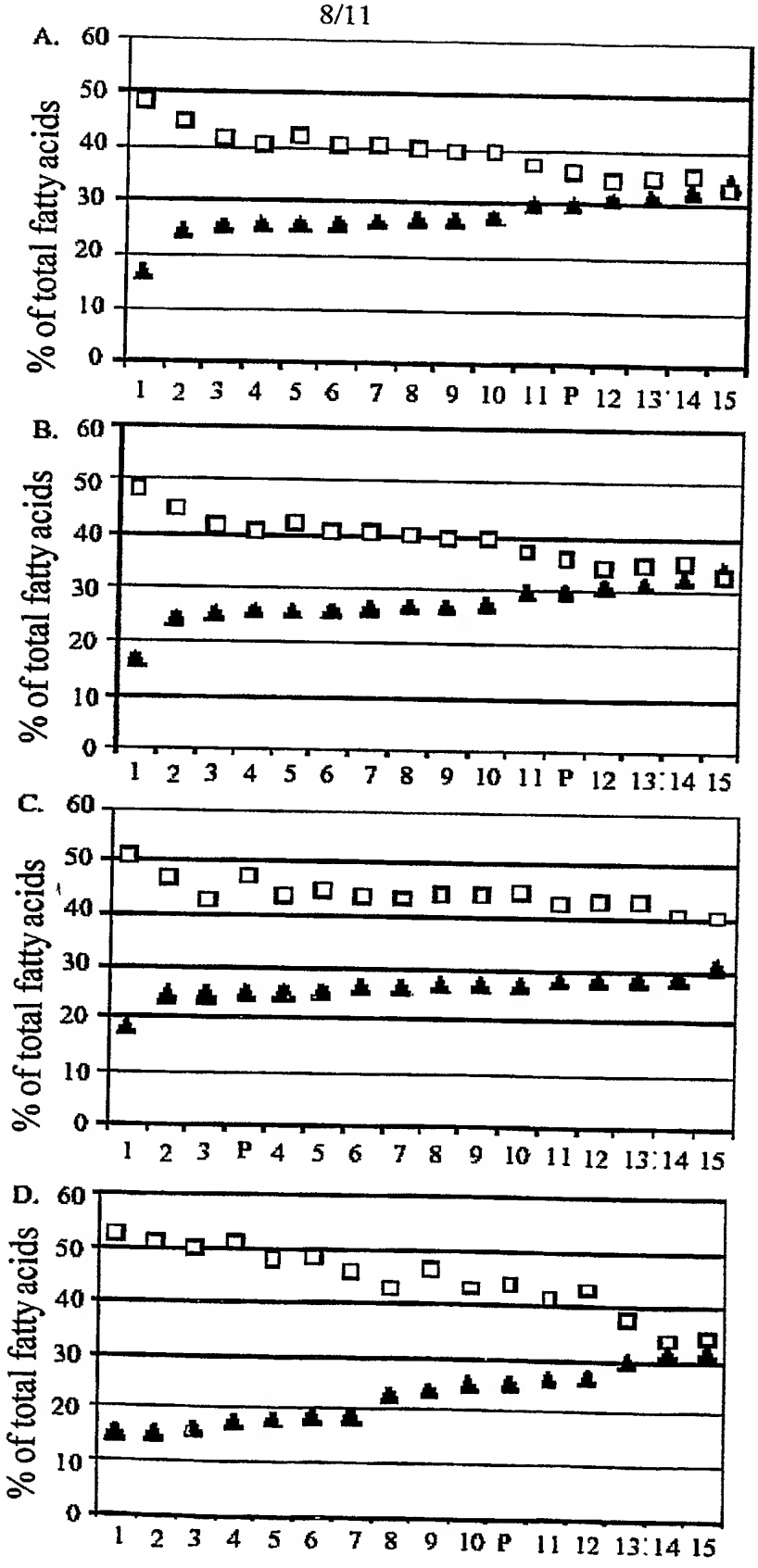
A.
B.
C.
D.
% of total fatty acids
0
10
20
30
40
50
60
1 2 3 4 5 6 7 8 9 10 11 P 12 13 14 15
1 2 3 P 4 5 6 7 8 9 10 11 12 13 14 15
1 2 3 4 5 6 7 8 9 10 P 11 12 13 14 15

FIG. 8

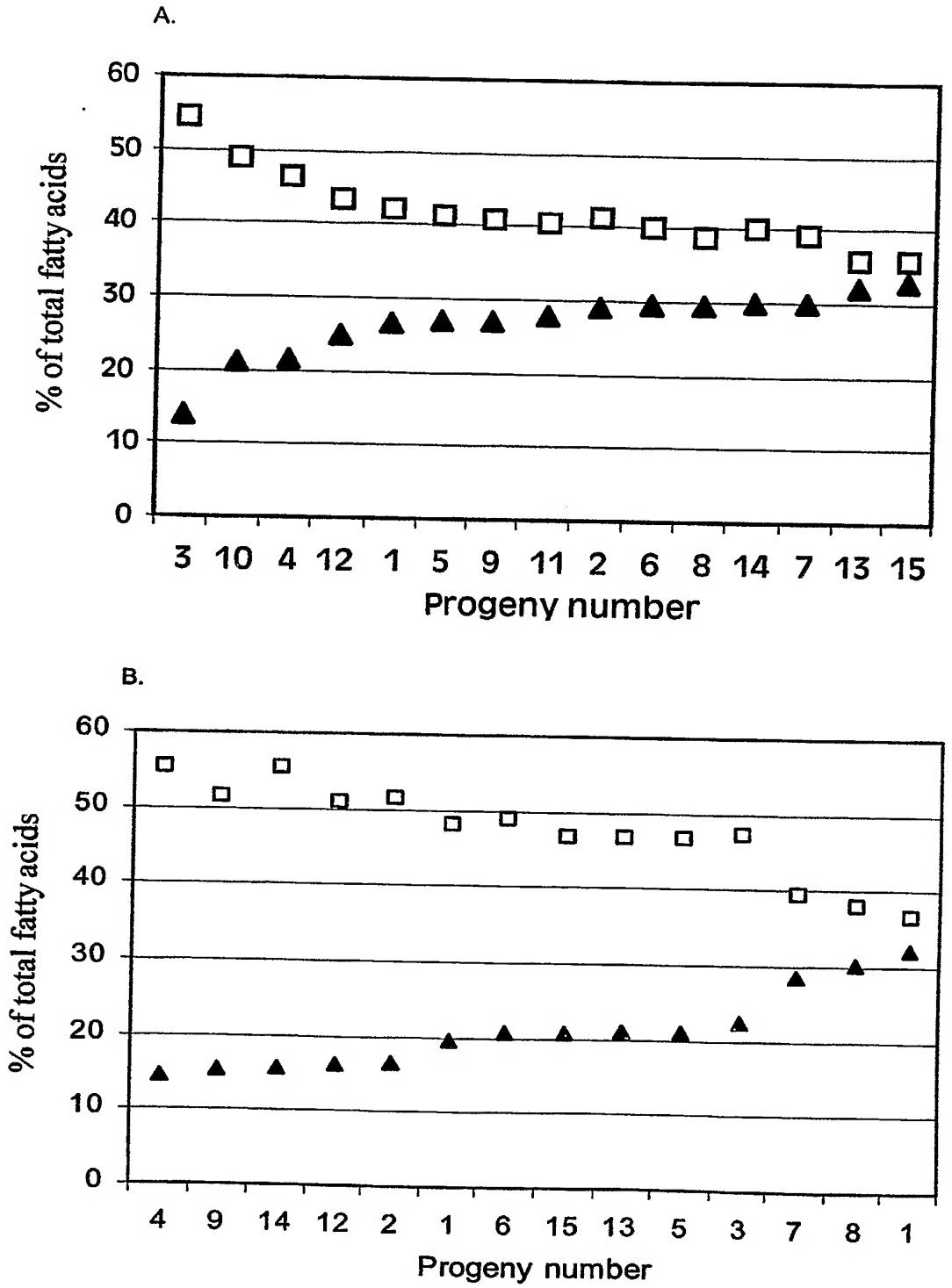
A.
% of total fatty acids
60
50
40
30
20
10
0
3 10 4 12 1 5 9 11 2 6 8 14 7 13 15
Progeny number
B.
% of total fatty acids
60
50
40
30
20
10
0
4 9 14 12 2 1 6 15 13 5 3 7 8 1
Progeny number

FIG. 9

```
ctattttcat cctatttaga aatccaagtt gacacctaaa atttagttgg actgccatgt 60

aggattatcg ttagagagat aacggagctt aacggtagag tgatcacttt gtaacaaaat 120

aataacaaaa gtgactaaag tgtaacattt caaacataaa tgattaaaat ataacctgag 180

gcaaacaaaa atgactattt ttatagatta ccctaaaatt aaagagtcat ggccctagcc 240

cctcgcctac ttgtttgttt ttaataaact aacatagtat aatatattgt taggattata 300

taaaattatt aataaatagt ataattaatt taaaat                          336
```

FIG. 10

ctattttcat cctatttaga aatccaagtt gacacctaaa atttagttgg actgccatgt 60

aggattatcg ttagagagat aacggagctt aacggtagag tgatcacttt gtaacaaaat 120

aataacaaaa gtgactaaag tgtaacattt caaacataaa tgattaaaat ataacctgag 180

gcaaacaaaa atgactattt ttatagatta ccctaaaatt aaagagtcat ggccctagcc 240

cctcgcctac ttgtttgttt ttaataaact aacatagtat aatatattgt taggattata 300

taaaattatt aataaatagt ataattaatt taaaatttat gaaaaataaa ttaccatatt 360

tcttaaatac gtggcacctt atgttggatt ggactgtata acttatatac tattatctat 420

attgaatcca aatccttact tttaagcgtt tttagtgaaa cattttattt tccattctta 480

ttatataaat ttatataatg atataatatg taatacttag ataatattat tgaaaaagaa 540

taaaaatacc tcaaactttg aaaggactaa tttgtatgag catcaaacgt acaggatacc 600

aaaagtatac atatctgaat ttgttcatat ctcctgcaac tcatagatca tcaccatgca 660

cagcaacatg tgtacacttg acttgtcctc tatcaactca acccttaact cagtgaatcg 720

ggacatctct gtctcacttt aaaacccttc ccagtttcaa cactctttga attcaactga 780

gttcacatac aacacaacac agtccatcat ctttctgctg ttaaagcatc atcatttcgc 840

cccttccagt tacagatgca acatgaaccc ccctgcaaca aagtttgtcc gaaccttgct 900

agtaccatgt gaagggatgt ggcatctcga tatctaccca ccactataca aaaaaaaaaa 960

aaagagacaa tatttcgtct tctttaattt gcacactcgt catcttgcat gtcaatgtct 1020

tcaacacgtt gatgaagatt tgcatgcaaa aatatcacct tccacagctc caccttctat 1080

aaatacatta ccactctttg ctattacc 1108

FIG. 11